A SECOND GENE WHICH AFFECTS THE RNA PROCESSING ENZYME RIBONUCLEASE P OF *ESCHERICHIA COLI*

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1. Introduction

The *Escherichia coli* RNA processing enzyme RNase P was shown to participate in processing of tRNA [1] and rRNA [2]. Mutations which affect this enzyme were isolated in [3,4]. Recently a mutation affecting RNase P, isolated [3] was mapped near min 82 of the *E. coli* chromosome [5]. This mutation was designated *rnpA49*. (For nomenclature of mutations affecting RNases see [6].) Here we shall describe the isolation of a second mutation which affects RNase P and show that it maps near min 68 of the *E. coli* chromosome [7].

2. Results and discussion

In an attempt to isolate mutants defective in RNA processing, temperature-sensitive mutants were isolated from an *rnc-105* strain [8,9]. One such mutant was defective in RNase E while another described here turned out to be defective in RNase P. (For further details about the isolation and the screening procedures see [8,9].)

During the screening of Ts^- strains isolated from the *rnc* mutant, the pattern of RNA observed at the nonpermissive temperature (43°C) suggested to us that one strain was defective in the enzyme RNase P. Since at the time we had constructed *rnc-105 rnpA49* double mutant strains [2] it was possible for us to compare the pattern of RNA synthesized in the suspected *rnc rnp* mutant strain (N3187) with that observed in constructed double mutant strains. Such strains are characterized by the appearance of a 19 S RNA molecule, which contains 16 S rRNA and spacer tRNA, and of a number of tRNA precursor molecules, and by the disappearance of the 4.5 S RNA molecule [2].

To ascertain whether strain N3187 contains an *rnp* mutation we transduced into it an *rnc*$^+$ gene. (For further details about such experiments see [2].) The resultant strain behaves very much like an *rnpA49* mutant. It is still temperature sensitive and the

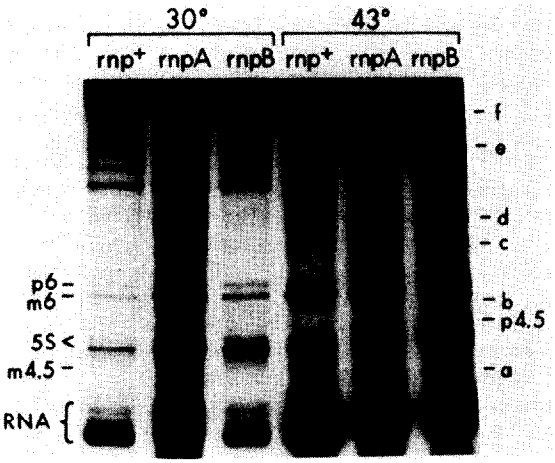

Fig.1. Electrophoretic analysis of RNAs from wild-type cells and *rnpA* and *rnpB* mutants. Cells were grown in a low phosphate medium [16] at 30°C. When they reached an A_{560} of 0.4 they were labeled with $^{32}P_i$ for 30 min or were transferred to 43°C and labeled with $^{32}P_i$ for 30 min, 40 min after the shift. The cultures were labeled with 40, 40 and 80 μCi/ml, respectively, at 30°C and with 40, 80 and 200 μCi/ml at 43°C. The cells were prepared for electrophoresis and cell lysates were applied to the slots of a 5%/10% thin polyacrylamide gel [2,16]. The top 5% part of the gel is not shown here. On the left the positions of a number of RNA moelcules are shown, and on the right a number of molecules are marked (a–f) which accumulate in *rnp* mutants and are not observed in *rnp*$^+$ cells (*rnp*$^+$, N2099; *rnpA49*, N2020, *rnpB3187*, N3854).

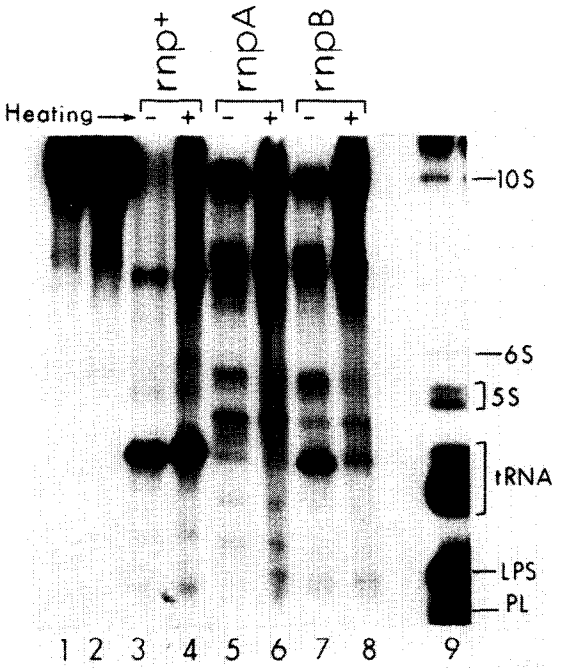

Fig.2. Processing of an RNA precursor by extracts from *rnp*$^+$, *rnpA* and *rnpB* cells. The strains used for this experiment were the same as those used in fig.1. The RNA precursor was isolated from an *rnpA* mutant labeled at 43°C with $^{32}P_i$ by the methods in [11,17]. Cell extracts were prepared from 600 ml cultures of cells grown at 30°C by grinding with alumina [18]. For heating, S30 extracts were incubated for 1 h at 47°C at 1 mg protein/ml. The assays were carried out according to [2]. They contained, in 50 μl, 100 μg protein and 250 000 cpm of substrate. The assays were incubated for 2 h at 37°C. About half of each reaction was applied to the polyacrylamide thin slab gel (5%/10%). After electrophoresis the gel was dried and the top 5% portion was removed prior to the autoradiography. (1) Unincubated substrate; (2) 2 h incubated substrate (without enzyme); (3–8) heated and unheated extracts from the three strains; (9) long term (5 h) labeled cells, (*rnp*$^+$) to supply markers; LPS, lipopolysaccharides; PL, phospholipids.

pattern of the RNA synthesized at 43°C is very similar to that found in an *rnpA49* strain (fig.1). At permissive temperatures, however, the strains carrying the *rnpB3187* mutation show an essentially normal pattern of RNA synthesis while *rnpA49* strains do not (fig.1). (Since as can be seen below, the mutation which affects the RNase P in strain N3187 is unlinked to the *rnpA49* mutation we designated this mutation *rnpB3187*.) A clear indication that the mutant is defective in RNase P comes from the observation that the small stable 4.5 S RNA does not appear in the mutant at the nonpermissive temperature, since this molecule was shown to be processed in vitro by RNase P [10].

To determine whether the mutant was defective in RNase P a number of RNAs which accumulate in an *rnpA49* mutant were isolated. They were subjected to digestion by a purified RNase P preparation (a generous gift from Dr S. Altman) and a molecule of ~10 S, which was processed to tRNA size material, was chosen for further investigation. (Previous studies showed that tRNA precursors, in this size range, accumulate in an *rnpA49* mutant at 43°C [11].) This RNA was digested by heated and nonheated extracts prepared from *rnp*$^+$, *rnpA*, and *rnpB* strains. The results of this experiment are shown in fig.2. They indicate that indeed the strain is defective in the enzyme RNase P. These results also suggest that the *rnpB* mutation affects a structural component of the enzyme, since the activity is thermolabile. As can be seen in fig.2 when the substrate was incubated by itself (lane 2) it was not degraded or processed. When the substrate was incubated with heated or nonheated cell extract from the *rnp*$^+$ strain, material accumulated in the 4 S region (lanes 3,4). On the other hand, when the *rnpB* mutant was used as the source of enzyme (lanes 7,8), while the nonheated extract produced 4 S material in quantities similar to those produced by the *rnp*$^+$ cell extracts, the heated extracts showed very little activity. As can be seen in lanes 5, 6, heated and nonheated extracts from the *rnpA49* had very low activity. This phenomenon is well known [2,12] and it can be seen in fig.1 that even in vivo *rnpA49* cells grown at 30°C do not have the normal levels of RNase P, since their RNA synthesis is abnormal.

To find out whether or not the *rnpA* and *rnpB* are closely linked, P1 bacteriophage was prepared from both strains and reciprocal transductions were carried out in which Ts$^+$ recombinants were isolated; they were comparable to the number of Ts$^+$ recombinants isolated when the donor was an *rnp*$^+$ strain. (The number of Ts$^+$ recombinants was normalized in the same experiment to the number of transductants for an unrelated auxotrophic marker.) These experiments therefore suggested that the *rnpA49* mutation and the *rnpB3187* mutation occur in two different genes.

In order to further explore the location of the *rnpB3187* mutation, an F-strain carrying it was crossed to a number of Hfr strains with different points of origin scattered around the *E. coli* chromosome (for more details about this type of analysis see [13,14]).

These experiments suggested that the *ts* mutation carried by the *rnpB* strain is located between min 64 and min 81 of the chromosome. This location is similar to the location suggested for one of the *rnp* mutations isolated in [4] offering a location on the basis of F′-mediated crosses. Different mutations in this region were used to test for cotransduction with the *rnpB* gene and cotransduction was observed with an *argG* mutation. When an *rnpB arg*$^+$ strain was the donor and an *rnp*$^+$ *argG* was the recipient and selection was carried out for Arg$^+$ transductants, out of 40 Arg$^+$ recombinants 6 were Ts$^-$ and Rnp$^-$ as revealed by analysis of their growth and of their RNA patterns (see fig.1), i.e., 15% cotransduction. In a reciprocal cross where selection was carried out for Ts$^+$ transductants, cotransduction frequency of 22% was found between *argG* and *rnpB* when 60 Ts$^+$ recombinants were analyzed. The pattern of RNA in the recipients which received the *rnpB3187* mutation was virtually undistinguishable from the pattern of the RNA molecules observed in the donor strain (see fig.1). These experiments suggest that the Rnp and the Ts are either phenotypes caused by a single mutation or by very tightly linked mutations.

The enzyme RNase P may contain two components, protein and RNA [15]. It is possible that each of the *rnp* genes corresponds to one of these components. Another possibility is that one of the genes codes for the protein and the other for an enzyme which can affect the RNA or the protein moiety of RNase P. Further studies will be required to distinguish between these two possibilities or to suggest a new one.

Acknowledgements

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